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Dear Joe:

I am returning the record that you sent me for revision. Actually there does not appear to be very much wrong with it. A few words are of course missing at the very beginning but they are only platitudinous remarks and can probably very well be overlooked. Whether you will want to have this record transcribed or not I don't know as probably most of the material was covered in our personal discussion.

I don't have all of the notes which I may have written up myself on our conversations and I am off again for a trip so I thought I would get this off first.

I will collect the paralysed and Fla⁻ strains that you asked for and will wait to hear from you concerning the chambers for the chemotaxis experiments.

Here are some of the references that you asked for. Probably the most recent general account of rotating colonies is by Murray and Elder in the J. Bacteriology 58:351,1949. He has quite a good bibliography from which you can trace much of the earlier literature. I suspect there have been some more papers since then which could be found under the species names mycoides, rotans or circulans. The reference by Alpatov to which Murray refers is a rather interesting paper as it claims that there is a basic stereochemical differentiation between the left and right-handed strains. This conclusion was based on the differential activity of mepacrine and other inhibitors, including as I remember quenidine. You can make what you will of such a system. It certainly is a curious phenomenon.

The work by Seaman on the acetylcholinesterase system of Tetrahymena is in J. Cellular and Comparative Physiology 37:309,1951. Dougherty's work on axenic cultivation of Rhabditis is in a series of papers, the last of which that I know of is in J. Parasitology 39:381,1953.

Sonneborn's work on Stenostomum dates to 1930 and is in the J. Exp. Zoology 57:57. There are two papers, the second on the effects of lead acetate.

You mentioned that you were going to look up Jennings on the behavior of the protozoa. I would also refer you to the two-volume series by Hutner and Lwoff on the physiology and biochemistry of the protozoa. I had in mind that an organism like Tetrahymena would be useful as an addition to the test organisms not only for motility experiments but for the whole gamut of antibiotic activity.

I know that you will want to make up your own mind about what is worth doing in the screening program and that one of the considerations will be the simplicity of the tests rather than the relevance of the phenomenon. What follows is just my own personal reaction, and is based rather largely on intuition.

First, I would not really see very much hope in getting anything promising in the restoration of motility. If you do go into this you will have to look out for genetics as well as physiological effects and be sure that any motility that you are

able to induce in these paralyzed and Fla⁻ strains is dependent on the agent that you add to the system. If you use a mixture of strains for the purpose of economy, you may have to watch out for the possible stimulation of the transduction among the different mutagenes represented. It is fairly obvious how you would detect this artifact.

The chemotaxis experiments in some ways are a little wilder but they just could hit something of general biological interest along the lines of the suppression of irritability. The inhibition of motility, I would judge to be quite a worthwhile line of activity as it might pick up fundamental curare forms and other end-organ inhibitors.

While at Syracuse I already raised the question of screening for compounds that would interfere with the uptake or metabolism of glucose. The more I think about this, the more natural a program this seems to be. Consider the applications of a useful agent. They would include the control of such conditions as dental carries, diabetes, as well as obesity. It seems to me that there is every prospect that such compounds can be found although I would imagine that the first set that should be looked at would be compounds related to glucose itself and this is a rather difficult area of organic chemistry. However, just such sugar derivatives are by no means infrequent constituents of the known antibiotics. The screening system for an anti-glucose should be fairly simple. One would set up an ordinary antibiotic assay but conduct it on an organism like E. coli K12 let's say, on a glucose agar, for example glucose EAB agar, or glucose with some other indicators. Such indicators might be the base MacConkey agar, or just an ordinary pH indicator, or possibly one might use triphenyltetrasolium chloride. Activity would then be indicated by a zone where the bacteria could grow, presumably on the peptone in the agar, but would not ferment the sugar. There might also be some fairly simple osmotic systems where one could detect the role of glucose in preventing or accentuating cytolysis and use this as a basis of quick screening for the penetration of the sugar. There is every reason to believe that a glucose blocked strain would be able to grow inasmuch as we already have glucose-negative mutants of Escherichia coli. I doubt very much that even if you obtain a compound with the desired activity it would be therapeutically useful as such but my hope is that it would give you the necessary biochemical lead to know just what you have to make in order to obtain the indicated results. The idea of looking for lipase inhibitors is by no means a bad one either and off hand I can't find a more recent paper by Mortimer Starr which gives some improvements on the method but there is an older one by Knaysi in J. Bacteriology, Nov. 1941. Off hand again, I don't know whether it would be advantageous to test your material this way or directly on pancreatic enzyme. I do hope you will give very serious consideration to the desirability of looking for anti-glucose.

A couple of other notions have occurred to me since I saw you, Joe, including the following: We now have quite simple systems by which we can detect the sexual fertility of Escherichia coli and this can be done on a plating basis. Goodness knows what use it would be but it should not be too difficult to set up a protocol for the detection of agents which (a) would inhibit the fertility but not the viability of Hfr strains of E. coli or (b) which would potentiate the limited fertility of F+ strains. Let me know if the idea behind this appeals to you sufficiently that you would like me to give you some sort of protocol but I repeat it can be done probably more easily even than your present tests for the induction of lambda.

Very similar to this suggestion is the one of looking for agents which will influence transduction by lambda. There is a paper in the January 1956 issue of Genetics, of which we don't have reprints yet, which gives the outline of that system. With what

we call them Hft lambda, it would be no trick at all to screen for reagents which would inhibit the transduction process. Let me say right away that previous attempts to get at the fragments which are included in the bacteriophage have been quite unsuccessful and even such reagents as X-rays and ultraviolet light have had practically no effect on transductional efficiency even at doses which appear to knock out all of the viral activity. From this point of view this system might be better than most anti-phage testing systems because it would at least screen for a minority of agents and those which had the most far-reaching or deep-seated effects on the phage. Again, let me know if you are interested enough for me to set up a detailed protocol. It would not be very difficult.

I don't know how far-afield my suggestions should go as I sensed that you were yourself rather less concerned with non-microbial systems. However, it seems to me that someone should give attention for screening for effects on <u>in vitro</u> antibody-antigen systems. I have in mind particularly agents which might prevent complimentary action on hemolysis or which might facilitate it. I really don't know very much of the background of this but it seems to me that I have heard somewhere that such compounds as sodium salicylate have effects in these directions but which are not drastic enough to be of direct use. I hardly need enlarge on the potential significance of reagents which can intervene in these reactions. It seems to me at least in principal it should be quite possible to forfend transfusion reactions by reagents of this type.

Excuse me, Joe, for the quality of this record. I'm afraid that something has happened to the microphone and that's partly responsible.

Another product worth looking for might be specific hemagglutinin or even hemolytic activity. I think it would be worthwhile testing them to see if they have the same effect on all of the major blood types. There have been primitive proposals on some plant materials as substitutes for natural iso-agglutinins.

So long for now, Joe.

Joshua

JL:jlg